

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0009] Figures 1A-1E show the full-length nucleotide sequence (SEQ ID NO:1) encoding the amino acid sequence of feedback-resistant pyruvate carboxylase, and the corresponding amino acid sequence (SEQ ID NO:2).
- [0010] Figure 2 shows the comparison of amino acid sequences between the wild-type pyruvate carboxylase, isolated from ATCC21253, and the feedback-resistant pyruvate carboxylase (SEQ ID NO:2), isolated from Deposit Number NRRL B-11474.
- [0011] Figures 3A-3B show the full-length nucleotide sequence (SEQ ID NO:3) encoding the amino acid sequence of feedback-resistant pyruvate carboxylase.
- [0012] Figure 4 shows the effects of various substrate concentrations on the pyruvate carboxylase activity in *C. glutamicum* ATCC 21253 and NRRL B-11474.
- [0013] Figure 5 shows the effects of aspartate concentration on the activity of pyruvate carboxylase in *C. glutamicum* ATCC21253 and NRRL B-11474.
- [0014] Figure 6 shows the effects of acetyl-CoA concentration on the activity of pyruvate carboxylase in *C. glutamicum* ATCC21253 and NRRL B-11474.

DETAILED DESCRIPTION OF THE INVENTION

- [0015] The present invention relates to variations of the polypeptide comprising the amino acid sequence which codes for the pyruvate carboxylase as shown in SEQ ID NO:19. Preferably, the variations of pyruvate carboxylase enzyme in the present invention contain at least one mutation which desensitizes the pyruvate carboxylase to feedback inhibition by aspartic acid. Such mutations may include deletions, insertions, inversions, repeats, and type substitutions. More preferably, the amino acid sequence mutation which desensitizes the wild-type pyruvate carboxylase enzyme (SEQ ID NO:19) to feedback inhibition comprises at least

one substitution selected from the group consisting of (a) methionine at position 1 being replaced with a valine, (b) glutamic acid at position 153 being replaced with an aspartic acid, (c) alanine at position 182 being replaced with a serine, (d) alanine at position 206 being replaced with a serine, (e) histidine at position 227 being replaced with an arginine, (f) alanine at position 452 being replaced with a glycine, and (g) aspartic acid at position 1120 being replaced with a glutamic acid. Still more preferably, the variation of the polypeptide encoded by the amino acid sequence of SEQ ID NO:19 contains more than one of the above-mentioned mutations. Most preferably, the variation of the polypeptide encoded by the amino acid sequence of SEQ ID NO:19 contains all of the above-mentioned mutations. As one of ordinary skill in the art would appreciate, the numbering of amino acid residues of a protein as used herein, begins at the amino terminus (N-terminus) and proceeds towards the carboxy terminus (C-terminus), such that the first amino acid at the N-terminus is position 1.

[0016] An embodiment of the present invention relates to an isolated or purified nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:2, (b) a nucleotide sequence which encodes the amino acid sequence of SEQ ID NO:4, (c) a nucleotide sequence encoding the amino acid sequence encoded by the DNA contained in Deposit Number NRRL B-11474 or (d) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), or (c).

[0017] Further embodiments of the invention include isolated nucleic acid molecules that comprise a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 97%, 98%, 99% or 100% identical, to any of the nucleotide sequences in (a), (b), (c) or (d) above, or a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c) or (d) above. However, the polynucleotide which hybridizes does

not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

[0018] Another aspect of the invention is directed to nucleic acid molecules at least 90%, 95%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 3 (SEQ ID NO:3) or to the nucleic acid sequence of the deposited DNA (NRRL B-30293, deposited May 30, 2000).

[0019] A further aspect of the invention provides a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17.

[0020] By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the pyruvate carboxylase polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence.

[0021] As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotide sequence of the deposited DNA can be determined conventionally using known computer programs such as the FastA program. FastA performs a Pearson and Lipman search for similarity between a query sequence and a group of sequences of the same type nucleic acid. Professor William Pearson of the University of Virginia Department of Biochemistry wrote the FASTA program family (FastA, TFastA, FastX, TFastX

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